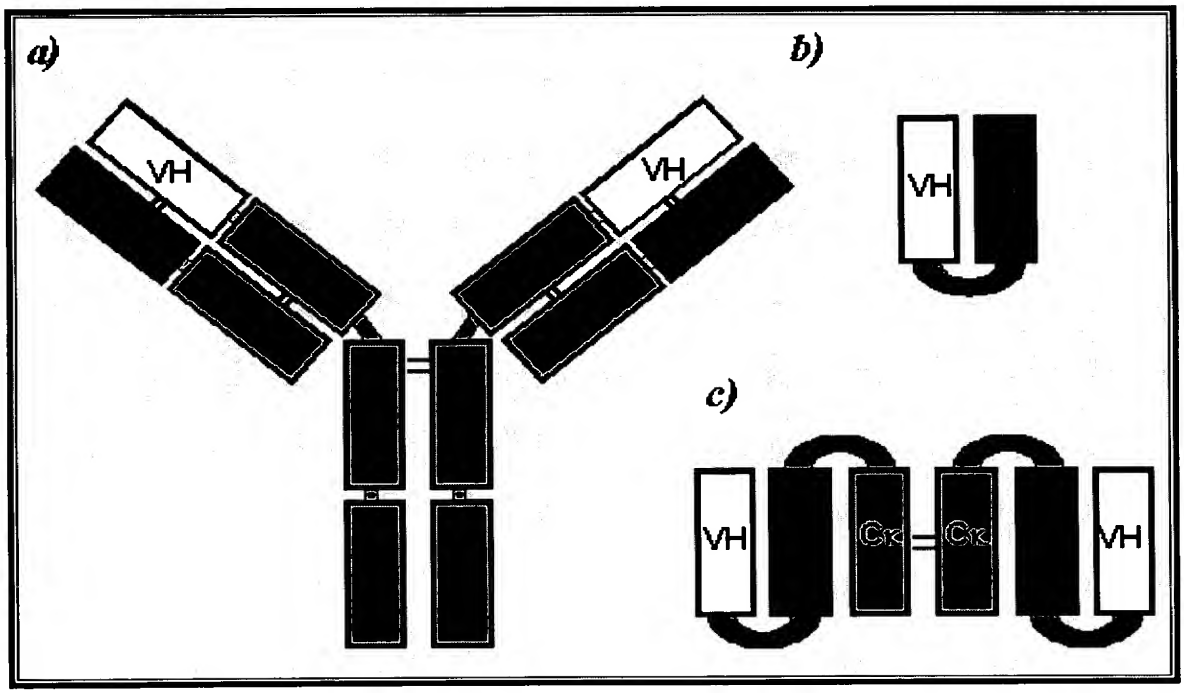
SINGLE CHAIN ANTIBODY VARIABLE REGION FRAGMENTS (scFv)

Naturally occurring antibodies (of isotype IgG) produced by B cells, consist of four polypeptide chains (*figure 1a*). Two heavy chains (composed of four immunoglobulin domains) and two light chains (made up of two immunoglobulin domains) are held together by disulphide bonds. The bulk of the antibody complex is made up of constant immunoglobulin domains (shown in green). These have a conserved amino acid sequence, and exhibit low variability. Different classes of constant regions in the stem of the antibody generate different isotypes of antibody with differing properties. The recognition properties of the antibody are carried by the variable regions (VH and VL) at the ends of the arms (shown in yellow and red). Each variable domain contains three hypervariable regions known as *complementarity determining regions*, or CDRs. The CDRs come together in the final tertiary structure to form an antigen binding pocket. The human genome contains multiple fragments encoding portions of the variable domains in regions of the immunoglobulin gene cluster known as V, D and J. During B cell development these regions undergo recombination to generate a broad diversity of antibody affinities. As these B cell populations mature in the presence of a target antigen, hypermutation of the variable region takes place, with the B cells producing the most active antibodies being selected for further expansion in a process known as *affinity maturation*.

A major breakthrough was the generation of monoclonal antibodies, pure populations of antibodies with the same affinity. This was achieved by fusing B cells taken from immunized animals with myeloma cells. This generates a population of immortal hybridomas, from which the required clones can be selected. Monoclonal antibodies are very important research tools, and have been used in some

therapies. However, they are very expensive and difficult to produce, and if used in a therapeutic context, can elicit and immune response which will destroy the antibody. This can be reduced in part by humanizing the antibody by grafting the CDRs from the parent monoclonal into the backbone of a human IgG antibody. It may be better to deliver antibodies by gene therapy, as this would hopefully provide a constant localized supply of antibody following a single dose of vector. The problems of vector design and delivery are dealt with elsewhere, but antibodies in their native form, consisting of two different polypeptide chains which need to be generated in approximately equal amounts and assembled correctly are not good candidates for gene therapy. However, it is possible to create a single polypeptide which can retain the antigen binding properties of a monoclonal antibody.

Figure 1

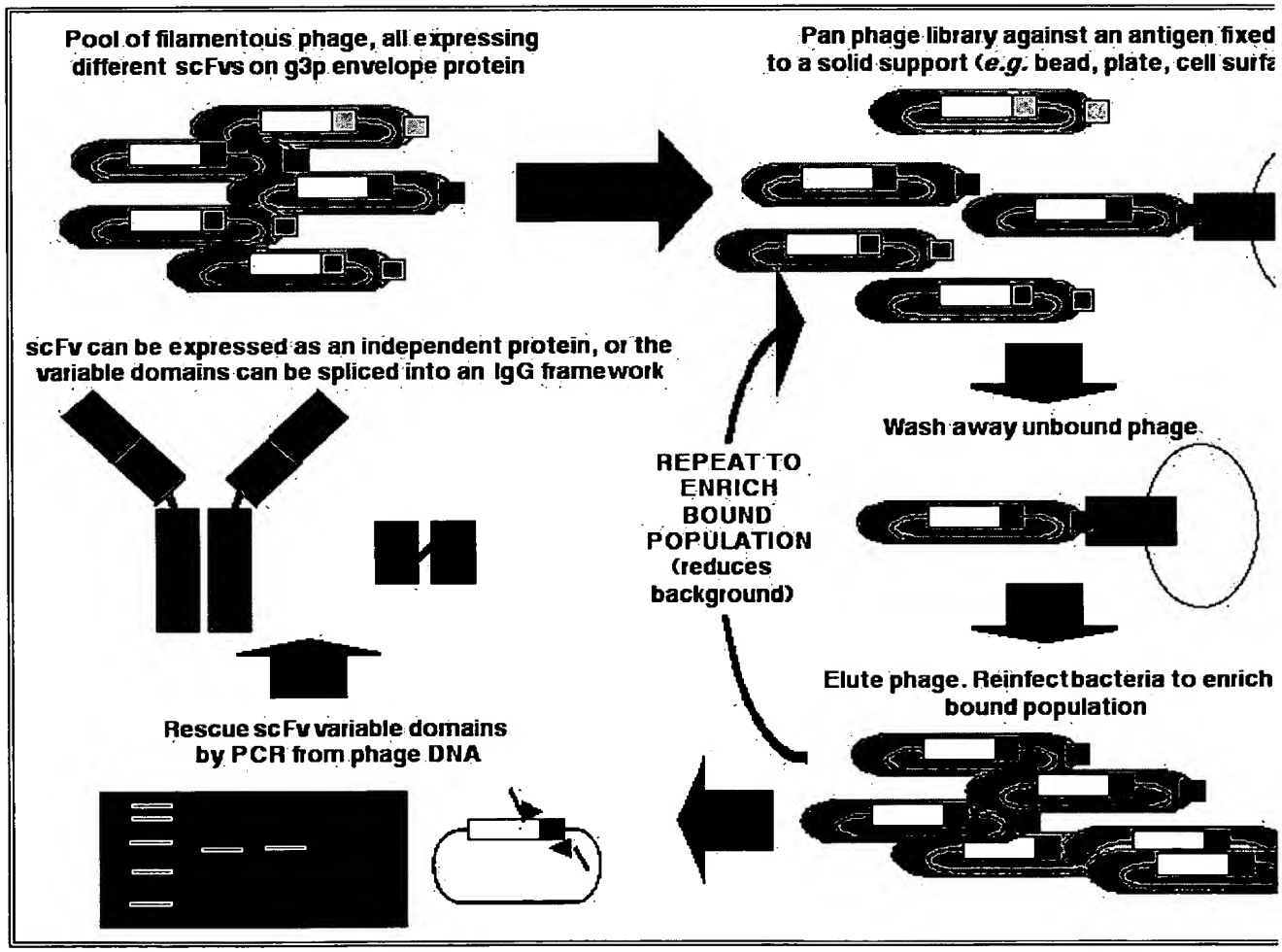


The variable regions from the heavy and light chains (VH and VL) are both approximately 110 amino acids long. They can be linked by a 15 amino acid linker with the sequence $(glycine_4serine)_3$, which has sufficient flexibility to allow the two domains to assemble a functional antigen binding pocket (*figure 1b*). Addition of various signal sequences allows the scFv to be targeted to different organelles within the cell, or to be secreted. Addition of the light chain constant region (Cκ) allows dimerization via disulphide bonds (*figure 1c*), giving

increased stability and avidity. However, there is evidence that scFvs spontaneously multimerize, with the extent of aggregation (presumably via exposed hydrophobic surfaces) being dependent on the length of the glycine-serine linker.

How are the variable regions obtained for constructing the scFv. If a monoclonal antibody against your target of interest is available, then it is a simple procedure to use RT-PCR to clone out the variable regions from mRNA extracted from the parent hybridoma. Degenerate primers targeted to the relatively invariant framework regions can be used. However, in many cases this option is not available, or it may be preferred to generate the scFv *de novo*. This can be done using phage display technology (*figure 2*).

Figure 2



The non-lytic phage M13 is a 900nm long, 6.5nm diameter filament with a single stranded circular DNA genome encoding

10 proteins. Capsid proteins g3p and g8p can present foreign protein sequences fused to their N-termini. Such domains include antibody fragments (scFv and Fab'), peptides, enzymes and enzyme inhibitors. How is phage display used to identify scFvs with unique binding properties? An scFv library, obtained either by PCR from genomic sequences for immunoglobulin genes randomised by CDR shuffling, or by inclusion of foriegn random sequences in the CDR regions, can be fused to the N-terminus of g3p. A pool of phage, all with different binding properties resulting from the scFv in the capsid is then generated by growth in bacteria. A phage particle in this library contains both phenotype (in the scFv domain in its capsid) and genotype (as the scFv-g3p fusion is encoded within the phage genome). Linkage of genotype and phenotype is vital in any library screen of this type. The phage library is screened for its ability to bind to an antigen of interest, which could be artificially bound to a solid support, or on the surface of a cell. Phage which binds to the target are rescued an expanded by further growth in fresh bacteria. This pool of selected phage can then be enriched through further rounds of selection. Eventually it will be possible to rescue individual clones which will hopefully have the desired binding properties. The scFv domain can then be pulled out from the phage by PCR and put into your protein expression system of choice.

WHAT CAN scFvs BE USED FOR?

There is a lot of interest in the possibility of using scFvs as therapeutic agents. The ability to generate scFvs with novel binding capacities in a much quicker, easier (and animal friendly!) way than monoclonal antibodies, coupled with scFvs lower immunogenicity given the lack of the Fc domain, makes them seem promising candidates.

THIS BIT IS STILL UNDER CONSTRUCTION!!!!!!

CANCER IMMUNOTHERAPY

One proposed method of treating cancer is to try to target the immune system to launch a cytolytic attack against the tumour. How can this be achieved? Although vaccines are being developed against tumour antigens, there are concerns surrounding the expression of these antigens on normal tissues (with the attendant risk of triggering a harmful autoimmune response)

HIV

INTRABODIES

ABZYMES

USEFUL ANTIBODY LINKS

Recombinant Antibody Links by Stefan Dubel. An extremely comprehensive list of links to all things to do with antibody engineering and antibody libraries.

Pluckthun Lab at the University of Zurich. Lots of stuff about directed molecular evolution of proteins and antibodies, and especially the development of ribosome display.

Martin Lab at the University of Reading, UK. Information about antibody structure. Also lots of useful links too.

Department of Immunotechnology , Lund University, Sweden. The Ohlin group's work on molecular breeding of antibodies by CDR shuffling.

Diversys . Ian Tomlinson and Greg Winter's new company, with information about their recombinant antibody technologies.

Morphosys has a recombinant human antibody library of complexity ~10^{10}. Information of how this technology works and how to obtain it.

MORE ANTIBODY LINKS COMING SOON

BACK

Minimal structural elements of an inhibitory anti-ATF1/CREB single-chain antibody fragment (scFv41.4).

Olsen RJ, Mazlo J, Koepsell SA, McKeithan TW, Hinrichs SH.
Hybrid Hybridomics. 2003 Apr;22(2):65-77

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Antibody variable domains represent potential structural models for the rational design of therapeutic molecules that bind cellular proteins with high affinity and specificity. The Activating Transcription Factor 1 (ATF1)/Cyclic AMP Response Element Binding Protein (CREB) family of transcription factors are particularly relevant targets due to their strong association with melanoma and clear cell sarcoma. Biochemical and structural investigations were performed to optimize a single-chain antibody fragment (scFv), scFv41.4, that disrupts the binding of ATF1/CREB to cyclic-AMP response elements (CRE) in vitro and inhibits transcriptional activation in cells. Molecular modeling and ligand docking simulations suggested that scFv41.4 could function as a disulfide-deficient single domain scFv. Functional studies verified that deletion of the light chain did not result in reduced inhibitory activity. The isolated heavy chain was predicted to assume a relaxed structural conformation that maintained a functional antigen binding pocket. The minimal structural elements necessary for intracellular function were further analyzed by selective deletion of CDR1 and CDR2. V(H)-CDR1 and V(H)-CDR3 were shown to play a key role in antigen binding activity, but V(H)-CDR2 was dispensable. Thus, scFv41.4 represents a unique molecule with potential for use in the design of peptidomimetic derivatives having therapeutic application to human cancer.

Analysis of antigen binding and idiotypic expression by antibodies with polyglycine-replaced complementarity-determining regions.

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J Immunol. 1996 Feb 1;156(3):1071-81

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We investigated the feasibility and usefulness, for structure-function studies, of removing the side chains of entire complementarity-determining regions (CDRs) of Abs by replacement with polyglycine. The CDRs of a murine Ab specific for p-azophenylarsonate (Ars) were replaced with polyglycine, one CDR at a time and in combinations, by oligonucleotide-directed mutagenesis of the V region genes. Mutant Abs were expressed in transfected hybridoma cells and analyzed for Ars binding and for idiotypic expression. The results suggest that, except for the longest CDRs, polyglycine replacement does not alter the general structure of the Ab molecule. However, for analysis of functional contributions of a CDR, the polyglycine replacement method appears to be most useful for CDRs with extended structures whose replacement by polyglycine does not affect the structure of other parts of the variable regions. In the current studies, such CDRs were CDR1 of the heavy chain (H1) and CDR2 of the light chain (L2). The polyglycine replacement of L2, which does not contain an Ag-contacting residue, allowed the formation of an Ars binding Ab. Furthermore, this mutant Ab revealed previously uncharacterized contributions of L2 to idiotypic expression. Polyglycine replacement of H1 abolished Ars binding as expected, because H1 contains an Ag-contacting residue. However, introduction of the contacting residue (Asn) on the polyglycine-replaced H1 background restored the ability of the Ab to bind Ars. The results suggest that polyglycine replacement of CDRs can provide structural information that complements and extends the information obtained by other methods.